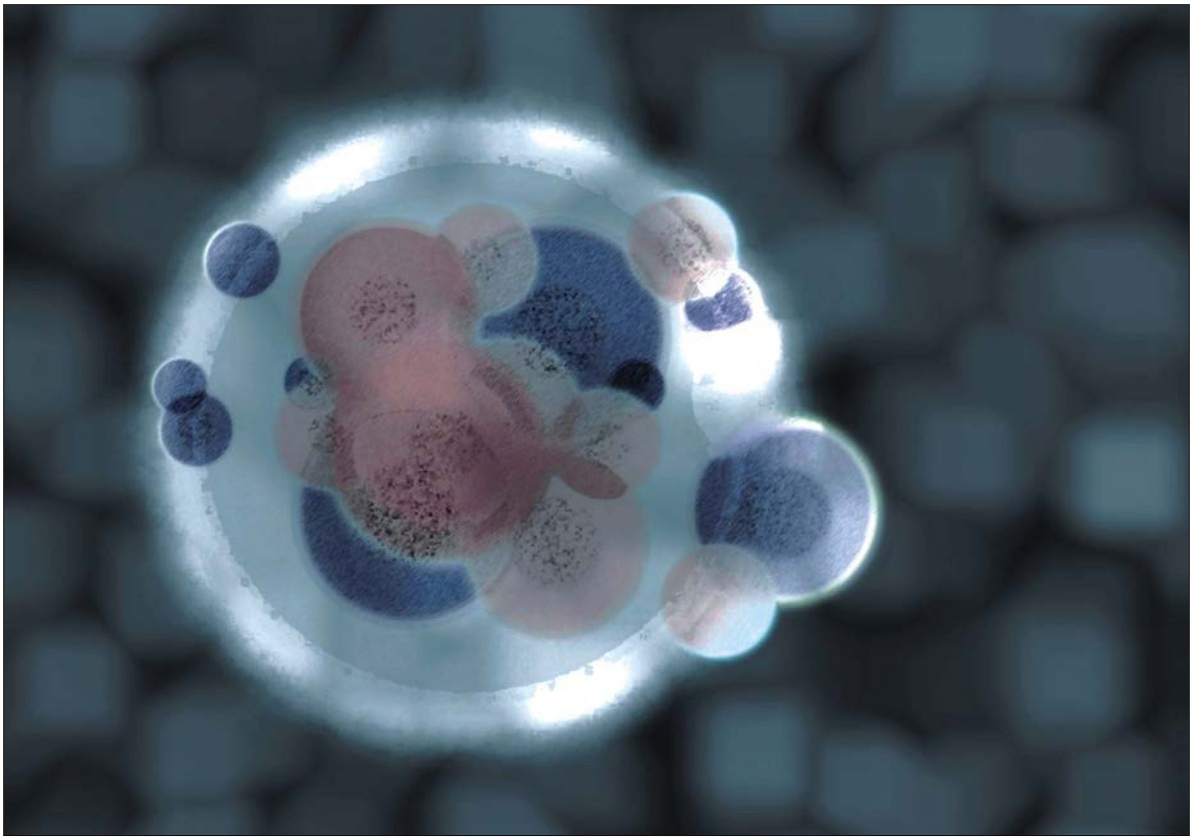

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Survival of Somatic and F-RNA Coliphages in Treated Wastewater Effluents and their Impact on Viral Quality of the Receiving Water Bodies in the Eastern Cape Province-South Africa

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Abstract: In the wake of growing suspicion of the implication of poor wastewater treatment in the Hepatitis A virus outbreak in the Eastern Cape Province, this study aimed to investigate the survival of viral indicators in treated effluent samples collected from four wastewater treatment plants of this province and to determine the impact of the effluents on the receiving water bodies. The concentration of free chlorine residuals and the viral quality of the effluents in terms of somatic and F-RNA coliphages were determined according to standard methods. The study revealed that although the average free chlorine residual concentration of the treated effluents was $\leq$0.32 mg L^{-1}, somatic and F-RNA coliphages were still found in the treated effluents. Viral quality of the treated effluent samples collected from Alice, Fort Beaufort, Dimbaza and East London wastewater treatment plants was very poor and did not complied with the recommended limits set by the official guidelines in South Africa in terms of coliphages, which are 0-1 pfu/100 mL for negligible risk for domestic water use and 0-20 pfu/100 mL for recreational water use. There were generally higher somatic coliphage counts (average ranges: 4.67 $\log_{10}$ to 6.39 $\log_{10}$ pfu/100 mL) than F-RNA coliphage counts (average ranges: 3.37 $\log_{10}$ to 5.26 $\log_{10}$ pfu/100 mL) in the treated effluents. In the receiving water bodies, the highest and the lowest counts of both somatic (average: 5.12 $\log_{10}$pfu/100 mL and F-RNA (5.58 $\log_{10}$pfu/100 mL) coliphages were recorded in Dimbaza and East London, respectively. The direct relationship between the poor viral quality of the treated effluents, the impact on the receiving water bodies and the consequent threat to the health of the surrounding communities calls for a modified approach in disinfection and proper management of wastewater treatment plants in the province.

Key words: Survival, coliphages, wastewater, effluent, receiving water body

INTRODUCTION

One of the objectives of recycling wastewater includes the removal of pathogenic microorganisms, thereby protecting the quality of water sources and reducing the cost of drinking water treatment. This objective has not yet been achieved in many parts of the world, especially in developing countries. Consequently, wastewater treatment plants discharge significant amounts of faecal pollution indicators and pathogenic micro-organisms into the receiving water bodies, leading to a reduction in the quality of the water sources (Bahlaoui *et al*., 1997; Simpson and Charles, 2000; Chau, 2002; Momba and Mfenyana, 2005). This is a matter of great concern as it is common for the rural communities to use the effluent and/or the receiving water bodies such as river, dam or Ocean Beach as a water source for drinking, bathing, washing (i.e., clothes, dishes etc.) or for recreational purposes (Momba and Mfenyana, 2005). The microbiological quality of these water sources could influence infection and disease in communities.

In South Africa, different species of pathogenic bacteria such as *Escherichia coli*, *Aeromonas hydrophila* and *Vibrio* species have been isolated from both the final effluents and the receiving water bodies of different wastewater treatment plants located in the Eastern Cape Province (Momba and Mfenyana, 2005). In January 2005, a Local New Paper (Daily Dispatch, 2005) reported the case of Hepatitis A virus outbreak in Buffalo Flats that led to the infection of eight children and the death of one boy in the province. The direct discharge of poor treated wastewater effluents into the receiving water bodies was then suspected as the major sources of this outbreak. The Daily Dispatch (2005) also suggested a link between outbreak and proper maintenance of the wastewater plants in the province.

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While bacterial indicators such as fecal coliforms are used to monitor the quality of the treated effluents, it has been reported that the water treatment and catchments management strategies based on bacteriological indicators do not provide the necessary protection against virus infections, meaning that viruses are more persistent than bacteria in water environments (Havelaar *et al*., 1993). Monitoring of the viral pathogens for routine control purpose is based on somatic and F-RNA coliphages, due to the fact that these indicators are relatively simple and more rapid to detect, easy to characterize in water and they are always present in the faeces of man and warm-blooded animals and hence in large numbers in sewages. Moreover, they share similar survival characteristics with enteric viruses when discharged into the aquatic environment (Hilton and Stotzky, 1973; Gajardo *et al*., 1991; Havelaar *et al*., 1993; Schwab *et al*., 1995; Abad *et al*., 1997).

Monitoring of the viral pathogens is impracticable for routine control purposes in South African rural wastewater treatment plants. However, monitoring sewage for pathogens has been demonstrated to be an excellent epidemiological tool for determining what diseases may be prevalent in the community at any one moment (Momba and Mfenyana, 2005). In the wake of growing suspicion of the implication of poor wastewater treatment in the Hepatitis A virus outbreak in the Eastern Cape Province, it was important to investigate the survival of somatic and F-RNA coliphages in treated effluents. The main objective of the present study was to determine the impact of the viral quality of the respective receiving water bodies from four wastewater treatment plants in the province, which may further influence viral infection and disease in the community. This study therefore intended to provide information that could assist the water authorities to address problems in the management of wastewater treatment plants in terms of viral standards of the effluent as set down by official guidelines in South Africa.

MATERIALS AND METHODS

Study sites: Four wastewater treatment plants that serve the Nkonkobe Municipal areas (Alice and Fort Beaufort) and the Buffalo City (Dimbaza and East London) in the Eastern Cape Province of South Africa were used in the present study. All of the plants in this study employ biological wastewater composed of an activated sludge system, followed by chlorination of the final effluent. The Alice and Fort Beaufort Sewage Works are located in rural areas, while the Dimbaza Sewage Works and the East Bank Reclamation Works (East London) are located in semi-urban and urban areas, respectively.

The Alice wastewater treatment plant is situated on the banks of the Tyume River, which is also used as the receiving water body for the final effluent from the plant. The final effluent from Fort Beaufort Sewage Works is discharged into the Kat River. The Dimbaza wastewater treatment plant discharges its final effluent into a stream that empties into the Tembisa sewerage dams. The final effluent from the East Bank Reclamation Works is discharged into the Indian Ocean between Nahoon and Eastern Beach at Bats Cave and into a pound for the irrigation of a nearly golf course. Supernatant liquor from the sedimentation tanks is channeled into a fish pond located within the plant premises. All these final effluents or receiving water bodies are often used by the communities for one or various purposes, which include domestic, agricultural or domestic use.

Sample collection: Sample collection was done on a monthly basis for a period of 11 months from October to November 2003 and from March to November 2004. Wastewater samples were collected from the raw influents, the treated effluents and the receiving water bodies of the above-mentioned wastewater treatment plants using sterile bottles. For the chlorinated final effluents used for microbiological analyses, the sampling was done with the sterile glass bottles containing ca 17.5 mg L^{-1}. The free chlorine residual concentrations of the final effluents were determined on-site using a multi-parameter ion specific meter (Hanna-BDH laboratory supplies).

The detection of somatic and F-RNA coliphages was done using internationally accepted techniques and principles. Briefly, the enumeration of somatic and F-RNA coliphages was performed on double-agar-layer plaque assay (SABS, 2001), using *Escherichia coli* strain C (ATCC 700078) nalidixic acid-resistant mutant WG5 and *Salmonella typhimurium* WG 49 nalidixic acid-resistant mutant as host respectively. The preparation of the wastewater samples, media and inoculums' cultures were prepared as described elsewhere (Grabow, 1996; Mooijiman *et al*., 2001).

Statistical analyses: All statistical analyses were carried out using the SPSS computer statistical software (version 11.0). A treatment means plot of the three variables-free chlorine concentrations, somatic coliphage and F-RNA coliphage counts-was produced as exploratory analyses. Logarithmic transformation of the counts was used as a way of stabilizing the error variances. The Pearson's correlation analysis were used to test for the linear association between the three variables. This was followed up with a one way analysis of variance

(ANOVA) for each type of organisms. Lastly, the Tukey's multiple comparison procedure was used to identify the significantly different pairs of means for the sites and the various zones. All tests were carried out at a 5% significance level.

RESULTS

Availability of Free chlorine residual concentrations in the final effluents: Table 1 and Fig. 1 show the concentrations of free chlorine residuals recorded in the final effluents of the four plants during the study period. In all the plants, the concentration of free chlorine residuals fluctuated throughout the study period. The Alice final effluents had a minimum free chlorine residual concentration of 0.24 mg L^{-1}, a maximum of 0.39 mg L^{-1} and an average of 0.33 mg L^{-1}. The final effluents collected from the Fort Beaufort wastewater treatment plant had a minimum free chlorine residual concentration of 0.32 mg L^{-1}, a maximum of 0.48 mg L^{-1} and an average of 0.39 mg L^{-1}. The minimum concentrations of the free chlorine residuals were 0.16 and 0.24 mg L^{-1} in the final effluents collected from the Dimbaza and the East London wastewater treatment plants, respectively. The maximum concentrations of the free chlorine residuals in the treated effluents of these two plants were 0.37 and 0.54 mg L^{-1}, while the average concentrations were 0.31 and 0.37 mg L^{-1}, respectively. The Fort Beaufort displayed the highest free chlorine concentrations, while the Dimbaza wastewater treatment plant showed the lowest concentrations. Moreover, no chlorine overdosing was recorded in the Fort Beaufort plant during the study period. The Alice and the East London final effluents had similar minimum free chlorine residual concentrations during November 2004 (results not shown).

Patterns and efficiency of the plants for the removal of coliphages: Table 2 and 3 indicate the profile of viral indicators from the influents to the receiving water bodies. In the influent samples, the Fort Beaufort wastewater treatment plants had the highest average count of 7.78 $\log_{10}$ pfu/100 mL somatic coliphages, followed by the Alice wastewater treatment plant (6.95 $\log_{10}$ pfu/100 mL)

Table 1: Profile of chlorine residual concentrations in the treated effluents of Alice, Fort Beaufort, Dimbaza and East London wastewater treatment plants from October 2003 to November 2004

Sampling site	Sampling stages	Minimum	Maximum	Mean	SD
Alice	Effluent	0.24	0.39	0.33	0.06
Fort beaufort	Effluent	0.32	0.48	0.39	0.06
Dimbaza	Effluent	0.16	0.37	0.31	0.08
East london	Effluent	0.24	0.54	0.37	0.11

No. of samples = 32

and the Dimbaza wastewater treatment plant (Table 2). The lowest average count was observed in the East London wastewater treatment plant (Table 2, Fig. 1).

With regard to the patterns and efficiency of each plant for the removal of viral indicators, data revealed a decrease in the number of somatic coliphages (Table 2) from the influents to the treated effluents. Although some variations were noted in somatic coliphage counts (maximum counts of 5.67 $\log_{10}$, 6.81$\log_{10}$, 5.76 $\log_{10}$ and 5.11 $\log_{10}$ pfu/100 mL in the effluents collected from Alice, Fort Beaufort, Dimbaza and East London, respectively), in general the removal efficiency of each plant was limited between 1.03 and 1.82 $\log_{10}$ pfu/100 mL somatic coliphages. Throughout the study period, this viral indicator was still present in the treated effluents of all the plants, with the exception of the East London wastewater treatment plant that showed a minimum somatic coliphage of 0 pfu/100 mL.

Results in Table 3 show that the maximum counts of F-RNA coliphages in the influents collected from East London, Dimbaza and Fort Beaufort wastewater treatments were above 6 $\log_{10}$ pfu/100ml although there were some variations from one plant to another. Compared

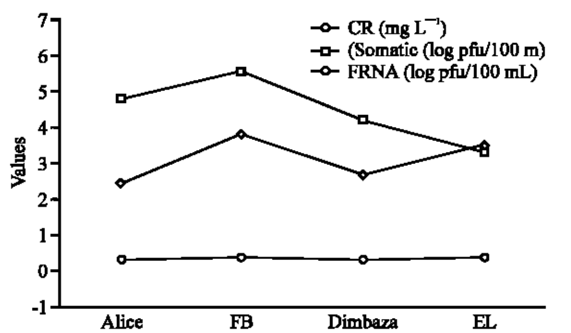

Fig. 1: Survival of somatic and F-RNA coliphages (Log_{10} pfu/100 mL) in treated wastewater effluents with an average free chlorine residual (CR) of 0.32 mg L^{-1}

Table 2: Profile of Somatic coliphages in the influents, treated effluents and receiving water bodies of Alice, Fort Beaufort, Dimbaza and East London Wastewater treatment plants

Sampling site	Sampling stages	Minimum	Maximum	Mean	SD
		------------(Log_{10} pfu/100 mL)			-----------
Alice	Influent	5.25	7.27	6.95	6.68
	Effluent	3.63	5.67	5.13	5.26
	Receiving body	2.10	5.06	4.40	4.71
Fort beaufort	Influent	2.48	7.78	7.42	7.42
	Effluent	2.80	6.81	6.39	6.48
	Receiving body	2.00	5.95	4.82	4.77
Dimbaza	Influent	4.20	7.04	6.64	6.62
	Effluent	2.63	5.76	5.11	5.40
	Receiving body	0.00	5.80	5.12	5.45
East london	Influent	5.16	6.41	6.11	6.25
	Effluent	0.00	5.11	4.67	4.74
	Receiving Body	0.00	4.70	4.05	4.34

No. of samples = 32

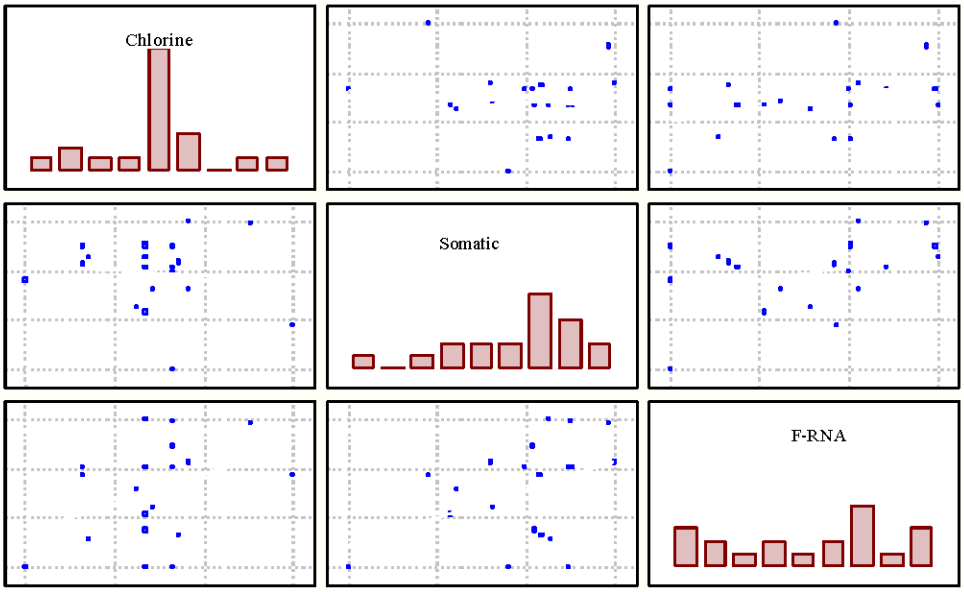

Fig. 2: The correlations matrix showing no linear association between the chlorine and somatic and F-RNA coliphage counts (in log_{10} pfu/100 mL)

to these three plants, the influent samples collected from the Alice wastewater treatment plant had the lowest F-RNA coliphages (5.83 log_{10} pfu/100 mL). A decrease in the number of F-RNA coliphages was noted from the influents to the treated effluents of each wastewater treatment plant. However, the removal efficiency of this viral indicator by each of these Sewage Works was only limited between 1.03 and 1.88 log_{10} pfu/100 mL.

Generally, there were higher counts of somatic coliphages in all samples collected from various wastewater treatment plants (Table 2, Fig. 1), except for the treated effluent samples collected from the East London wastewater treatment plant (Table 3). In this plant, the counts of F-RNA coliphages were slightly higher than those of somatic coliphages. The highest counts for both types of organisms were recorded in the treated effluents collected from Fort Beaufort. The lowest somatic and F-RNA coliphage counts were observed in the effluent samples from East London and Alice, respectively (Table 2, 3, Fig. 1). A comparison of the efficiency of the plants for the removal of both types of viral indicators confirmed that the Fort Beaufort Sewage Works had the lowest average coliphage removal of 1.03 log_{10} puf/100 mL while the highest average coliphage removal was found in Alice Sewage Works (average removal of 1.82 log_{10}/100 mL for somatic coliphages and 1.88 log_{10}/100 mL for F-RNA coliphages). Statistical evidence showed no linear association between the free chlorine concentrations and the counts of both somatic and F-RNA coliphages (Fig. 1, 2) as all p-values exceeded 0.05 according to Pearson's correlation analyses. However, there seemed to be a weak linear association between somatic and F-RNA coliphages counts.

Table 3: Profile of F-RNA coliphages in the influents, treated effluents and receiving water bodies of Alice, Fort Beaufort, Dimbaza and East London Wastewater treatment plants (No of samples: 32)

Sampling site	Sampling stages	Minimum	Maximum	Mean	SD
		------------(Log_{10} pfu/100 mL)-----------			
Alice	Influent	3.39	5.83	5.25	5.46
	Effluent	1.05	4.19	3.37	3.87
	Receiving body	4.36	5.36	5.10	4.87
Fort beaufort	Influent	4.17	6.78	6.26	6.39
	Effluent	0.00	5.90	5.23	5.57
	Receiving body	0.00	5.58	4.92	5.23
Dimbaza	Influent	2.14	6.89	5.54	6.58
	Effluent	0.00	4.83	4.20	4.47
	Receiving body	0.00	6.54	5.58	6.19
East london	Influent	0.00	6.99	6.32	6.63
	Effluent	0.00	5.94	5.26	5.59
	Receiving body	0.00	3.92	2.59	2.94

Impact of the survival of viral indicators in treated effluents on the received water bodies: For viral indicator profiles of the receiving water bodies, the highest and the lowest counts of both somatic (average: 5.12 log_{10}pfu/100 mL) and F-RNA (5.58 log_{10}pfu/100 mL) coliphages were recorded in water samples collected from the Dimbaza and East London receiving water bodies, respectively (Table 2, 3, Fig. 1). To determine the significantly different pairs of sites and zones, the Tukey's multiple comparison procedures revealed that the viral indicator counts depended on sampling sites, zones and type of organisms. The least significant differences were found to be 3.49 and 2.75 for the sites and the zones respectively. Since, there was a significant organism effect, the ANOVA test with log counts of coliphages as response and sites and zones (effluents and receiving water bodies) as predictors was carried out for each organism separately. Significant differences were found between the effluents and the receiving water bodies with higher somatic coliphage counts in the effluents than in the receiving water bodies ($p \leq 0.05$). However, the sites were not significantly different in terms of somatic counts. With the exception of the samples collected from the Alice plant where higher F-RNA coliphage counts were recorded in the receiving water body than in the effluent (a phenomenal indication of contamination from other sources outside the treatment plant system), statistically no significant difference was found between the counts of the F-RNA-coliphages in effluents and those in the receiving water bodies for other plants.

DISCUSSION

Worldwide, 2.4 billion of people are without access to adequate sanitation facilities and the vast majority of those people reside in developing nations (WHO/UNICEF, 2000). In Africa for example, the 34 million people who have gained access to a sewer connection over the 1990s represent only 20% of the new African population, which gives an estimate of 169 million people (WHO/UNICEF, 2000). Despite access to improved sanitation systems, it is important to bear in mind that most of the wastewater collected through the sewer systems are inadequately treated and are discharged directly into rivers, lakes and oceans where it used by communities for drinking, recreational and agricultural purposes (Momba and Mfenyana, 2005). This situation is applicable to the Eastern Cape Province where a large proportion of the population (68%) lived below the South African national poverty line in 2002 (UNDP, 2004) and approximately 11 and 38% of the population lived in informal and traditional structures, respectively. Piped water distribution had reached only 62% of the households and 31% of the households had no toilet facilities (SSA, 2003).

The present study revealed important findings on the survival of viral indicators in the treated effluents discharged in the received water bodies used by the communities of the Buffalo City and Nkonkobe Municipality in the Eastern Cape Province. The capacity of the four wastewater treatment plants to provide effluents of high quality should depend on the performance of each functional unit in the plant, which mainly included anaerobic zone, aerobic zone, clarifier and the disinfection of the effluents.

It is well known that the availability of the free chlorine residuals in the final effluent gives an indication of the efficiency of the plants for the removal of pathogenic microorganisms (Pretorius and Pretorius, 1999). To comply with the South African guidelines, water that is used for domestic purposes must have the free chlorine concentrations ranging between 0.3 and 0.6 mg L^{-1} for an ideal free chlorine residual concentration and between 0.6 and 0.8 mg L^{-1} for a good free chlorine residual concentration DWAF (1998). The results of this study revealed that only the treated effluent samples collected from Fort Beaufort had free chlorine concentrations that were within the recommended limits throughout the study period (Table 1). Although, the East London had a maximum free chlorine residual concentration that was within the recommended limits, a minimum free chlorine concentration below these limits (Table 1) disqualified this plant to comply with the required standards. Even though an average of 0.32 mg L^{-1} free chlorine residual concentration was recorded in each plant, coliphages were still found in the treated effluents (Table 1-3, Fig. 1). According to Tree *et al.* (2003), the inactivation of somatic and F-RNA coliphages is dose rather than disinfectant residual dependent. During the study period, the initial coliphage counts in the influent samples were determined (Table 2-3), but the wastewater operators were not aware of the initial chlorine doses used for the treatment of the final effluents. Nevertheless, the results of this study clearly demonstrated that wastewater treatment reduced the number of viral indicators from the influents, but the complete removal of coliphages was not achieved.

The present study is a clear picture of the situation in most poor areas of developing countries. The viral indicators in final effluents revealed that all the four wastewater treatment plants were not efficient in removing coliphages up to the acceptable standards, which are 0-1 pfu/100 mL for negligible risk for domestic water use and 0-20 pfu/100 mL for recreational water use (DWAF, 1996). During the study period, the four wastewater treatment plants were characterized by poor operational state and inadequate maintenance, i.e. design weaknesses, overloaded capacity, faulty equipment and machinery which might interfere with the performance of the plants in the removal of coliphage. This resulted in major pollution problems and impacted on the quality of water resources, with river (Alice), dam (Dimdaza) and marine (East London) water quality standards consequently not meeting the regulatory standards.

Statistically, the pattern of the results gave an indication that the final effluents from all the plants contributed to the presence of coliphages in the receiving water bodies, although the Alice case showed that other sources might influence the pollution of the receiving water bodies (Fig. 1, 2). The results of this study confirms a previous investigation done by Momba and Mfenyana (2005) on the impact of inadequate treatment of wastewater on the bacteriological quality of the receiving surface water bodies in the Eastern Cape Province of South Africa. According to these researchers, the presence of coliforms in the final effluents of the Fort Beaufort, Alice, Dimbaza and East London wastewater treatment plants had a direct influence on the bacteriological quality of their respective receiving water bodies. The relationship was such that the better the quality of the final effluent, the better the quality of the receiving water bodies (Momba and Mfenyana, 2005).

The overall sanitary qualities of all the receiving water bodies in terms of viral indicators were above the recommended limits and unacceptable for domestic or recreational use. These findings indicated that all the receiving water bodies might pose a threat to the surrounding communities that use these water sources. Taking into consideration the concentrations of the viral indicators in the treated effluents and their respective receiving water bodies, the results of the present study support the report by the Local Newspaper Daily Dispatch that stated in 2005 the culpability of these water sources in the Hepatitis A virus outbreak in the Eastern Cape Province.

The effect of exposure of the general population to wastewater aerosols from sprinkler irrigation of partially-treated wastewater has been reported in Israel, Hong Kong and in the United States of America. In Israel for example, there were increases in salmonellosis, shigellosis, typhoid fever and infectious hepatitis in the kibbutz population living near sprinkler irrigated field when the quality of water had 10^{6-8}TC/100 mL (Katzenelson *et al*., 1976; Chau, 2000). In another study, a two-fold excess risk of clinical enteric disease in young children living within 600 to 1000 m of the sprinkler irrigated field was found when the wastewater quality was very poor (10^{6-8}TC/100 mL) (Fattal *et al*., 1986). In USA, the Lubbock Infection Surveillance study measured the frequency of clinical viral infections (through reported illness supplemented by detection of viruses in stool samples) in a population before and after exposure to sprinkler irrigation with treated wastewater (Camann and Moore, 1987). This study revealed an association between high aerosol exposure and viral illness when low quality wastewater effluent from a trickling plant was used for irrigation; this association was of borderline significant ($p = 0.06$). These studies clearly demonstrate the importance of a proper management of wastewater treatment plants and also a regular monitoring of the quality of wastewater effluents and receiving water bodies that can therefore become an important tool to trace the origin of epidemic in a country.

CONCLUSION AND RECOMMENDATIONS

The disinfection method that is being practiced in South Africa in terms of chlorine residual concentrations was found not to be effective for the removal of somatic and F-RNA coliphages. Although an average chlorine residual concentration of 0.32 mg L^{-1} was found in various plants, viral indicators such somatic and F-RNA coliphages were detected in treated effluents. The poor viral quality of receiving water bodies was attributed to the presence of the coliphages in the effluents of the wastewater treatment plants. The outcome suggests that a new approach to disinfection which involves the use of more powerful disinfectants (such as a combination of chlorine and monochloramine) for the treatment of the effluents should be considered. This study also highlights the need to address the other contributing factors related to inadequate treatment of wastewater in the Eastern Cape Province. Thus, this study recommends equal attention to address the contributing factors affecting the quality of the receiving water bodies:

- A regular monitoring of the wastewater effluents and their respective receiving water bodies to trace the origin of epidemic in the province
- A proper management of wastewater treatment in terms of operational conditions of the plants and maintenance of the equipment and machinery
- The protection of water sources and the identification of outside sources of pollutions such as disposal of human and animal excreta by the local residents. For a proper management of faecal contamination of water sources in the Eastern Cape Province, the supply of sanitation facilities to rural communities is needed

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